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Biochimica et Biophysica Acta 1408 (1998) 290–295

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

Review

Interactions of surfactant protein D with pathogens, allergens and phagocytes

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Received 13 March 1998; received in revised form 8 June 1998; accepted 8 June 1998

Abstract

Surfactant protein D (SP-D) is considered to play an important role in innate immunity in the lungs by binding, via its multiple C-type lectin domains, to carbohydrate structures present on a range of viruses, bacteria, yeasts and fungi. The resulting agglutination of the target pathogens provides host defence which can be further enhanced by killing and clearance mechanisms mediated by phagocytic cells which carry receptors for SP-D. Recent findings suggest that SP-D, and the structurally related lung surfactant protein A (SP-A), may also modulate allergic reactions by binding certain glycosylated allergens. The finding of SP-D at a variety of other sites besides the lungs, such as the gastric mucosae, is suggestive that it may play a general protective role in several secretions.

Keywords: Lung surfactant; Innate immunity; Collectin; Surfactant protein D; C-type lectin

Contents

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0925-4439/98/$ – see front matter
PII: S0925-4439(98)00074-X

1. Introduction

The four well-characterised lung surfactant proteins SP-A, SP-B, SP-C and SP-D appear to play quite different roles in the lung. SP-B and SP-C are hydrophobic polypeptides which are intimately associated with the lipid bilayer and are essential for full lung function via their property of accelerating the adsorption of lipids to the air-liquid interface. SP-A and SP-D are, in comparison to SP-B and SP-C, relatively hydrophilic and are members of the 'collectin' group of C-type lectins which include the serum proteins mannose binding lectin, conglutinin and collectin-43 [1]. The collectins are characterised by their having polypeptide chains containing a short (7–28 amino acids), cysteine-rich, N-terminal section followed by a collagen-like region (59–171 amino acids), then an α-helical section of approximately 37 amino acids and a C-terminal, C-type lectin domain (of about 120 amino acids). In SP-D three identical 43 kDa polypeptide chains form a 140 kDa trimeric subunit which contains a collagen-like triple helix (over the 171 residues of collagen-like sequence) and an α-helical coiled-coil (over the 37 residues of the α-helical section). Four of the trimeric subunits are linked via their N-terminal sections to yield a tetrameric 560 kDa molecule with 12 C-type lectin domains which can all potentially bind to arrays of carbohydrates of the type that can be found on the surfaces of certain lung pathogens. It is well-established that the serum collectins play an important role in innate immunity, via their carbohydrate recognition properties, and it has become clear, over approximately the past 5 years, that both SP-A and SP-D are also probably involved in host defence mechanisms. More extensive discussion on the structure and expression of SP-D is given in the article by E.C. Crouch [2] in this special issue of Biochimica Biophysica Acta. In the case of SP-A, although it is strongly associated with the surfactant lipids and has been proposed to play a role in surfactant homeostasis, it has been found that genetically engineered SP-A-deficient mice have essentially normal lung structure and function but are more susceptible to lung infections [3] and this area of research is more fully covered in the article by Korfhagen et al. [4] which is also presented in this special issue. There is a growing body of evidence which indicates that the primary role of SP-D in the lung is to combat infection and the production of genetically engineered SP-D-deficient mice should allow a full assessment of this point.

2. Interactions of surfactant protein SP-D with pathogens

It is well established that SP-D binds, in a calcium-dependent manner, to carbohydrate structures on the surfaces of a wide range of pathogens resulting in agglutination of the target organisms. The pathogens recognised and, in most cases, agglutinated by SP-D include Gram-negative bacteria [5], viruses [6], yeasts [7] and fungi [8].

The carbohydrate structures, on these pathogens, which are recognised by SP-D are in most cases not well characterised. However, in the case of SP-D interaction with Gram-negative bacteria the binding seems likely to be mediated between the C-type lectin domains of SP-D and the core sugars of the bacterial capsular lipopolysaccharides [5,9]. The strong inactivation by SP-D, of red blood cell haemagglutination activity of various strains of influenza A viruses, appears to result from the binding of the C-type lectin domains of the SP-D to carbohydrate structures on the head region of the virus haemagglutinin [6]. This view is supported by a report showing that the degree of glycosylation of a virus is an important factor in its virulence and also in the ability of SP-D to inhibit viral infection [10]. The structure recognised by SP-D when it binds and agglutinates the acapsular form of the yeast pathogen *Cryptococcus neoformans* is not known, but it is clear that the encapsulated form of the yeast is not recognised by SP-D and therefore the 1,3-mannose backbone and xylosyl and glucuronosyl side chains of the polysaccharide are not bound by SP-D [7]. The target for SP-D on the fungus *Pneumocystis carinii* is a heavily glycosylated 95–120 kDa cell surface molecule, the major surface antigen on the organism, which is rich in mannose, glucose and *N*-acetylglucosamine [11]. In the agglutination of the fungus *Aspergillus fumigatus* by SP-D it is not known exactly which structures are the binding sites for SP-D on the fungal conidia, but it has been established that SP-D binds strongly to two of the major anti-

gens, the glycoproteins gp45 and gp55, secreted by the fungus [12].

The recent finding [13] of exceptionally low levels of SP-D, and SP-A, in the bronchoalveolar lavage of children with cystic fibrosis may be very relevant to the fact that these patients are very susceptible to infection by a range of organisms, notably *Staphylococcus aureus*, *Haemophilus influenza* and *Pseudomonas aeruginosa*. However, it is not absolutely clear yet whether the low levels of SP-D and SP-A are brought about by a state of chronic infection in older children or whether there is a fundamental deficiency of SP-D and SP-A function in newly born children with cystic fibrosis which exposes them to infection. However, there is some evidence supporting the view that the low levels of SP-A may be related to chronic infection. This emerged from a study where very young children with cystic fibrosis, who had been identified by a neonatal screening programme, were examined and it was found that those with lung infections had increased SP-A levels in their bronchoalveolar lavage samples [14].

3. Cell surface receptors for SP-D

SP-A and other collectins such as serum MBL, but not SP-D, have been shown to bind to the widespread cC1qR/collectin 'receptor' [15] which now appears to be generally accepted as being a membrane-associated form of the intracellular, calcium-binding protein called calreticulin [16]. The binding of C1q, SP-A and MBL, but again not SP-D, to another molecule defined as a C1q receptor, the C1qRp present on cells of myeloid origin, platelets and endothelial cells, has also been demonstrated [17]. It does therefore appear unlikely that SP-D binds to cells via proteins which are known to show affinity for C1q and this is consistent with the demonstration that the specific binding of labelled SP-D to alveolar macrophages could be inhibited by unlabelled SP-D but not by C1q [18]. Assessment of the binding of SP-D to alveolar macrophages is complicated by the observation in one study that it bound specifically to the cells in a calcium-independent manner [18] yet in another study the binding was found to be calcium-dependent and inhibitable by saccharides [19]. In the study, where calcium-independent binding was seen, human SP-D and human alveolar macrophages were used whereas the other study, showing calcium-independent binding, used rat alveolar macrophages. It is possible that alveolar macrophages may even express three distinct types of binding protein/receptor for SP-D since the putative receptor, designated glycoprotein-340 (gp-340) has recently been characterised [20] and it appears to bind SP-D in calcium-dependent manner which is not inhibitable by saccharides. The gp-340 was isolated as soluble protein from lung lavage obtained from several alveolar proteinosis patients. It is composed of polypeptide chains of 340 kDa, when examined on SDS-PAGE under reducing conditions, and partial amino acid sequence showed that it is a new member of the scavenger-receptor cysteine-rich superfamily containing multiple scavenger receptor type B domains. The soluble form of gp-340 has an apparent molecular mass of approximately 1000 kDa in non-dissociating conditions and immunohistochemical analysis, using both polyclonal and monoclonal antibodies, has shown it to be present on the surface and within alveolar macrophages [20]. Although some partial cDNA data are now available it is still not known how the surface form of gp-340 is anchored in the membrane. It is possible that gp-340 is found as alternatively spliced soluble and membrane forms as has been described, in the mouse, for a closely related member of this scavenger-receptor family of molecules [21]. It has been shown that there is direct binding between gp-340 and purified native SP-D at physiological ionic strength and that the binding is dependent upon the presence of calcium but cannot be inhibited by maltose [20]. Similar binding is seen between gp-340 and a recombinant fragment of SP-D, composed of the trimeric α-helical neck region and three carbohydrate recognition domains. Although the interaction between gp-340 and SP-D involves the carbohydrate recognition domains of SP-D, the inability of maltose to block the interaction indicates that the binding between gp-340 and SP-D is not dependent upon the recognition of carbohydrate structures. Thus, gp-340 may represent one type of receptor for SP-D on alveolar macrophages but, as judged from the binding experiments described at the beginning of this section, there may be at least two other types still to be characterised.

4. Biological effects triggered by binding of SP-D to pathogens and to cells

SP-D has been shown to be a strong chemoattractant for both monocytes and neutrophils [22,8]. Since the chemotactic effect can be blocked by saccharides, or the addition of antibodies raised against the C-type lectin domains of SP-D, it is probable that the chemotactic effects are mediated by the C-type lectin domains of SP-D binding to cell-surface carbohydrate structures. SP-D has also been shown to enhance the production of oxygen radicals by alveolar macrophages and neutrophils [23,8]. An increased release of oxygen radicals from neutrophils was seen on the binding of SP-D to the conidia of *Aspergillus fumigatus* [8]. There is, at present, little evidence to show that binding and agglutination of pathogenic microorganisms by SP-D leads to increased phagocytosis of the pathogens. For example, although it was shown that SP-D enhanced the binding of *Pneumocystis carinii* to alveolar macrophages, in a rat model of infection, this did not enhance the uptake of the microorganism by the cells [11]. However, increased killing and phagocytosis of the conidia of *Aspergillus fumigatus* by neutrophils, on the addition of SP-D, has been reported [8].

5. Interactions of surfactant proteins SP-A and SP-D with allergens

The concept that SP-A and SP-D might play a role in the modulation of allergic responses stems from an observation in 1993 that SP-A binds to a variety of pollens which are common allergens, these included pollen from Lombardy poplar, Kentucky blue grass, cultivated rye and short ragweed [24]. In this study the SP-A was shown to bind to the major 57 kDa and 7 kDa proteins present in a water extract of Lombardy poplar pollen, and the results presented were consistent with the view that the interaction took place between the C-type lectin domains and carbohydrate structures on the pollen proteins. The binding of SP-A to the pollen grains allowed adhesion of the grains to the A549 cell line derived from alveolar type II cells. The possible involvement of SP-D, as well as SP-A, in allergen binding was examined in a study [25] involving allergens present in the faecal pellets of the house dust mite (*Dermatophagoides pteronyssinus*, *Der p*). The house dust mite is a major source of inhaled allergens, many of which are proteinases. Allergen-specific IgE antibody responses are seen against three major allergens (*Der p* I, II and III) as well as several minor allergens in allergic patients. It was found that both SP-A, and SP-D, bound to whole mite extract and purified native *Der p* I in a carbohydrate specific and calcium-dependent manner [25]. Furthermore both SP-A and SP-D were shown to inhibit allergen-specific IgE from binding to whole mite extract. This inhibition of binding appears to be functionally significant since it has recently been found that SP-A and SP-D can inhibit histamine release in an assay in which samples of diluted whole blood of asthmatic children were challenged with *Der p* allergen in the presence of surfactant proteins [26]. In the same study [26] SP-A and SP-D were shown to reduce the incorporation of [^{3}H]thymidine into peripheral blood mononuclear cells which had been isolated from asthmatic children and then stimulated with *Der p* extract or phytohaemagglutinin *p*. It therefore appears that SP-A and SP-D may modulate the development of asthmatic symptoms by both inhibiting histamine release in the early phase of allergen challenge and suppressing lymphocyte proliferation in the later states of asthmatic attacks where there is bronchial inflammation. These inhibitory effects, shown by SP-A and SP-D, against *Der p* allergen-mediated reactions may prove to be a general defence role which the lung surfactant proteins play against a range of glycosylated allergens. Support of this view is provided by the finding that SP-A and SP-D can inhibit specific IgE binding to the allergens of the fungus *Aspergillus fumigatus* and also block allergen-induced histamine release from allergic patients' basophils [12].

6. Evidence for the presence of SP-D at other sites in the body besides the lungs

SP-D is predominately localised in the lung, where it is synthesised by type II alveolar cells and Clara cells, but there is now convincing evidence that it is also synthesised and secreted at a variety of other sites in the body. The finding, by Northern blotting,

of a 1.5 kb signal for human SP-D RNA in samples from small intestine, colon, heart and pancreas, as well as lung [27], is consistent with the reports of mRNA for SP-D in the gastric mucosa of the rat [28], mouse heart, stomach and kidney [29] and mesentery cells in rat and man [30]. There is also good immunochemical evidence that SP-D protein is produced in the gastric mucosa in the rat [28] and also in human salivary, sweat, mammary and tear glands [31].

The finding of SP-A expression by epithelial cells of both the large and small intestine of the rat [32], and the presence of SP-A in human colon [33], suggests that SP-A, like SP-D, may also fulfil a role in innate immunity at other sites besides the lungs.

7. Expression of a recombinant fragment of SP-D for possible therapeutic use

A recombinant fragment of human SP-D, which is composed of the α-helical neck region and three C-type lectin domains, has been produced in *Escheirchia coli* and in the yeast *Pichia pastoris*. The approximately 60 kDa trimeric structure, which is held together by strong hydrophobic forces within the neck region of SP-D [34], has been shown to bind, in the same fashion as intact SP-D, to the lipopolysaccharides of several strains of Gram-negative bacteria known to cause lung infections [9]. This recombinant material has also been shown to be effective in blocking the interactions of glycosylated allergens with specific IgE [25,26]. The effectiveness of the recombinant trimeric neck-C-type lectin structure, derived from SP-D, in combating lung infection and allergen-mediated reactions is currently being tested in mouse models.

8. Conclusion

SP-D and SP-A appear to play important roles in innate immunity in the lung surfactant and perhaps at a number of other sites in the body. The recognition of pathogens by SP-D involves the binding of its trimeric clusters of carbohydrate recognition domains to arrays of complex carbohydrates on the surfaces of the target microorganisms. This action may simply agglutinate and neutralise the microorganisms but the reports of at least two types of specific receptors for SP-D on phagocytic cells is consistent with the view that triggering of cell-surface receptors by SP-D enhances the killing and clearance of lung pathogens.

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